post-translocated state under the energy bias, Tyr639 side chain is stabilized IN the active site in order to screen incoming nucleotides for pre-insertion. We further show kinetically that the screening at the early stage of nucleotide selection is particularly important for achieving both low error rate and high enough transcription speed. To test our hypotheses and examine the system with substantial details, atomistic molecular dynamics simulations are conducted to evaluate free energy changes accompanying essential conformational changes of the RNAP. We also study how exactly the nucleotide selection proceeds in T7 RNAP by examining binding affinities of different nucleotides to the elongation complex at both the pre-insertion and insertion checkpoints. Our studies intend to illustrate at multiple time and length scales the physical mechanisms of how a simplest RNAP moves and transcribes with high enough fidelity.

[1] A small post-translocation energy bias aids nucleotide selection in T7 RNA polymerase transcription. Jin Yu, George Oster. Biophysical Journal, 102, 532-541,2012

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Electron Microscopy-FRET Imaging Localizes the C-Terminus of the Second TFIIF Subunit in RNA Polymerase II-TFIIF with Nanometer Precision

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By tightly associating with RNA polymerase II (RNAPII), TFIIF a general transcription factor consisting of Tfg1 and Tfg2 subunit, plays dual roles in both initiation and elongation. Here, we employ a hybrid method of combining nano-probe electron microscopy (EM) and Förster resonance energy transfer (FRET) to investigate the probe-labeled C-terminus of Tfg2 in the RNAPII-TFIIF complex. Single particle EM analysis of nano-gold labeled RNAPII-TFIIF maps the C-terminus of Tfg2 inside the DNA binding cleft or close to the Rpb1 clamp. The FRET distance constraints in-between the pair of Tfg2 and Rpb4, and that of Tfg2 and Rpb2, confines the C-terminus of Tfg2 to the ridge of the Rpb1 clamp. Further FRET measurements followed by nano-positioning analysis reveal that, in the RNAPII-TFIIF elongation complex, the mean position of the C-terminus of Tfg2 shifts to the top of the Rpb1 clamp while, reciprocally, the transcription start site alters its position in the presence of TFIIF. Our results are consistent with the previous studies by crosslinking, establishing that Tfg2 is a malleable protein, and suggesting a dynamical role of Tfg2 in promoter clearance in transcription.

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Multiple RNA Polymerase Transactions on Single DNA Templates

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We are studying the interactions of more than one RNA polymerase (RNAP) operating on single linear double-stranded DNA templates using imaging by atomic force microscopy (AFM) [1,2]. These templates include a short polynucleotide sequence of A, C, G or T forming a single-stranded end loop to discriminate polarity of the DNA molecules in the AFM [3]. Model templates include convergently and tandemly-aligned promoters for bacterial *E. Coli* RNAP.

Measurement of the positions of the RNAP on the DNA before and after firing from the promoters allow us infer outcomes of ''collision'' events. The end-labelling ssDNA loop not only acts as a fiducial marker but also captures RNAPs that have fired from promoters directed towards and closest to the loop. This enables us to quantify different outcomes of these events between more than one RNAP operating in a convergent or tandem configuration. Interestingly, RNAPs which approach each other often stall before close contact is made, implying that transient DNA super-coiling between RNAP mediates their interactions. Furthermore, the distribution of outcomes for head-on (convergent transcription) and rear-end (tandem transcription) collision events are similar, suggesting that long range interactions mediated through the local DNA conformation serve to regulate RNAP activity and positioning on the templates. These experiments inform the regulation of genetic expression at the molecular level, including acting as simple models for nested genes.

[1] Billingsley D.J., Bonass W.A, Crampton N., Kirkham J. and Thomson N.H. (2012) *Physical Biology* 9 021001.
[2] Crampton N., Bonass W.A., Kirkham J., Rivetti C. and Thomson N.H. (2006) *Nucleic Acids Research* 34 (19) 5416-5425.
[3] Billingsley D.J., Crampton N., Kirkham J., Thomson N.H., Bonass W.A (2012) *Nucleic Acids Research* 40 (13) e99.

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An RNA Polymerase Finds its Promoter without Sliding along DNA

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Sequence-specific DNA binding proteins must quickly bind to their target sequence despite the enormously larger amount of non-target DNA present in cells. RNA polymerases (or associated general transcription factors) are hypothesized to reach promoter sequences by a facilitated diffusion (FD) mechanism. In FD, a protein first binds to non-target DNA and then reaches the target by a one-dimensional sliding search. We tested whether *Escherichia coli* σ^{54} RNA polymerase reaches a promoter by FD using a new variant of the CoSMoS multi-wavelength single-molecule fluorescence colocalization microscopy technique. The experiments directly compared the rates of initial polymerase binding to and dissociation from promoter and/or non-promoter DNAs measured in the same sample under identical conditions. Binding to a non-promoter DNA was much slower than to a promoter-containing DNA of the same length, indicating that the observed sequence non-specific binding is not on the pathway to promoter binding. Truncation of one of the DNA segments flanking the promoter to a length as short as 7 bp, or lengthening it to $>$ 3000 bp, did not significantly alter the rate of promoter-specific binding. The rate of polymerase binding to a single promoter sequence is equivalent to that observed for binding to $>$3000 bp of non-promoter DNA. These results exclude FD over distances larger than ~7 bp from playing any significant role in promoter search and also exclude other mechanisms (e.g., ''hopping'') that are mediated by flanking DNA. Instead, the data support a direct binding mechanism in which σ^{54} RNA polymerase reaches the promoter by simple three-dimensional diffusion through solution, and they suggest that binding is accelerated by recognition of atypical structural or dynamic features of DNA within the promoter sequence.

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The Mechanics of Initial Transcription: The RNA-DNA Hybrid Pushes on the Protein, and the Protein Pushes Back!

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RNA polymerases must couple the energetics of nucleotide addition to drive timed release of strong promoter contacts. RNA polymerases also undergo substantial structural changes in transitioning from sequence-specific initial transcription to stable elongation. Initially transcribing complexes are characteristically unstable, yielding short abortive products, but polymerase mutations have been isolated that dramatically reduce abortive instability. Understanding these mutations is essential to understanding the energetics of initial transcription and promoter clearance. using fluorescence probes of structural changes, we demonstrate that the low-abortive P266L mutant T7 RNA polymerase also transitions to elongation at *longer* lengths of RNA. We propose that both properties derive from a weakening of the initial barrier to hybrid-driven rotation of the promoter binding N-terminal platform, a motion necessary to drive promoter release. While the hybrid pushes on this protein element, the protein in turn pushes back on the hybrid, leading to the observed instability during initial transcription. Parallel biochemical experiments on bacterial RNA polymerase show that the sigma region 3.2 linker (analogous to the eukaryotic RNA polymerase ''B-finger'') functions in a very similar manner. Thus the mechanical ''pushing'' of the RNA-DNA hybrid against the protein is met by a reciprocal, and destabilizing, pushing of the protein against the hybrid.

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Single-Molecule Insights on Human RNA Polymerase II Transcription Regulation: Assembly of the Pre-Initiation Complex and the Roles of Activators

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Transcription of all protein-coding genes in human cells begins with the assembly of the RNA polymerase II (Pol II) pre-initiation complex (PIC) composed of more than forty polypeptides (total size of ~ 3MDa). Due to the high complexity and the dynamic nature of the PIC, the mechanism of its assembly and regulation remains elusive after decades of conventional biochemical studies. We have developed a surface-based, promoter-specific Pol II transcription system suitable for single-molecule imaging (Genes and Development 2012,